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Magnetic Resonance Spectroscopy following Mild Traumatic Brain Injury: A Systematic Review and Meta-Analysis on the Potential to Detect Posttraumatic Neurodegeneration

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Abstract: **INTRODUCTION:** Traumatic brain injury (TBI) is the most relevant external risk factor for dementia and a major global health burden. Mild TBI (mTBI) contributes to up to 90% of all TBIs, and the classification "mild" often misrepresents the patient's burden who suffer from neuropsychiatric long-term sequelae. Magnetic resonance spectroscopy (MRS) allows in vivo detection of compromised brain metabolism although it is not routinely used after TBI. **OBJECTIVE:** Thus, we performed a systematic review and meta-analysis to elucidate if MRS has the potential to identify changes in brain metabolism in adult patients after a single mTBI with a negative routine brain scan (CCT and/or MRI scan) compared to aged- and sex-matched healthy controls (HC) during the acute or subacute postinjury phase (≤ 90 days after mTBI). **METHODS:** A comprehensive literature search was conducted from the first edition of electronic databases until January 31, 2020. Group analyses were performed per metabolite using a random-effects model. **RESULTS:** Four and 2 out of 5,417 articles met the inclusion criteria for the meta-analysis and systematic review, respectively. For the meta-analysis, 50 mTBI patients and 51 HC with a mean age of 31 and 30 years, respectively, were scanned using N-acetyl-aspartate (NAA), a marker for neuronal integrity. Glutamate (Glu), a marker for disturbed brain metabolism, choline (Cho), a marker for increased cell membrane turnover, and creatine (Cr) were used in 2 out of the 4 included articles. Regions of interests were the frontal lobe, the white matter around 1 cm above the lateral ventricles, or the whole brain. NAA was decreased in patients compared to HC with an effect size (ES) of -0.49 (95% CI -1.08 to 0.09), primarily measured in the frontal lobe. Glu was increased in the white matter in 22 mTBI patients compared to 22 HC (ES 0.79; 95% CI 0.17-1.41). Cho was decreased in 31 mTBI patients compared to 31 HC (ES -0.31; 95% CI -0.81 to 0.19). Cr was contradictory and, therefore, potentially not suitable as a reference marker after mTBI. **CONCLUSIONS:** MRS pinpoints changes in posttraumatic brain metabolism that correlate with cognitive dysfunction and, thus, might possibly help to detect mTBI patients at risk for unfavorable outcome or posttraumatic neurodegeneration early.

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Magnetic Resonance Spectroscopy following Mild Traumatic Brain Injury: A Systematic Review and Meta-Analysis on the Potential to Detect Posttraumatic Neurodegeneration

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Keywords

Mild traumatic brain injury · Neuronal density/integrity · Glutamate · Magnetic resonance spectroscopy · Posttraumatic neurodegeneration · Metabolites · N-acetyl-aspartate

Abstract

Introduction: Traumatic brain injury (TBI) is the most relevant external risk factor for dementia and a major global health burden. Mild TBI (mTBI) contributes to up to 90% of all TBIs, and the classification “mild” often misrepresents the patient’s burden who suffer from neuropsychiatric long-term sequelae. Magnetic resonance spectroscopy (MRS) allows in vivo detection of compromised brain metabolism although it is not routinely used after TBI. **Objective:** Thus, we performed a systematic review and meta-analysis to elucidate if MRS has the potential to identify changes in brain metabolism in adult patients after a single mTBI with a negative routine brain scan (CCT and/or MRI scan) compared to aged- and sex-matched healthy controls (HC) during the acute or subacute postinjury phase (≤ 90 days after mTBI).

Methods: A comprehensive literature search was conducted

from the first edition of electronic databases until January 31, 2020. Group analyses were performed per metabolite using a random-effects model. **Results:** Four and 2 out of 5,417 articles met the inclusion criteria for the meta-analysis and systematic review, respectively. For the meta-analysis, 50 mTBI patients and 51 HC with a mean age of 31 and 30 years, respectively, were scanned using N-acetyl-aspartate (NAA), a marker for neuronal integrity. Glutamate (Glu), a marker for disturbed brain metabolism, choline (Cho), a marker for increased cell membrane turnover, and creatine (Cr) were used in 2 out of the 4 included articles. Regions of interests were the frontal lobe, the white matter around 1 cm above the lateral ventricles, or the whole brain. NAA was decreased in patients compared to HC with an effect size (ES) of -0.49 (95% CI -1.08 to 0.09), primarily measured in the frontal lobe. Glu was increased in the white matter in 22 mTBI patients compared to 22 HC (ES 0.79 ; 95% CI 0.17 – 1.41). Cho was decreased in 31 mTBI patients compared to 31 HC (ES -0.31 ; 95% CI -0.81 to 0.19). Cr was contradictory and, therefore, potentially not suitable as a reference marker after mTBI.

A.E. and M.H.-S. contributed equally to this work.

Conclusions: MRS pinpoints changes in posttraumatic brain metabolism that correlate with cognitive dysfunction and, thus, might possibly help to detect mTBI patients at risk for unfavorable outcome or posttraumatic neurodegeneration early.

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Introduction

Traumatic brain injury (TBI) is the most relevant external risk factor for dementia and shares hallmarks of neurodegenerative diseases, such as Alzheimer and Parkinson disease, and frontotemporal dementia [1–3]. Each year, over 50 million people sustain a TBI, which renders TBI the global leading cause of death and disability among the young and working populations. Thus, TBI is a major public health concern with tremendous socioeconomic implications and estimated costs of approximately USD 400 billion annually [4].

Mild TBI (mTBI) accounts for 80–90% of all brain impacts and is defined according to the American Congress of Rehabilitation Medicine (ACRM) by a score of 13–15 on the Glasgow Coma Scale (GCS), a maximum loss of consciousness of 30 min, and posttraumatic amnesia <24 h after the brain injury [5–7]. The term *mild TBI* often misrepresents the patient's burden with functional, emotional, and cognitive long-term sequelae, as half of mTBI patients with a negative cranial computed tomography (CCT) complain functional limitations 12 months after the brain impact, and up to one third suffer from cognitive dysfunction [8–12]. Nearly 90% of CCT or routine MRI brain scans remain negative despite major clinical health concerns with the inability to return to work after mTBI [13–17], which emphasizes the need for additional diagnostic tools to identify patients earlier who are at risk for unfavorable outcome or even posttraumatic dementia. Current state-of-the-art CCT and MRI techniques are limited in recognizing the full complexity of the mTBI neuropathology, and thus more sensitive, yet still realistic clinical measures to detect early compromised brain metabolism that might help to predict long-term cognitive deficits are required [16, 18].

Magnetic resonance spectroscopy (MRS) allows for in vivo measurement of metabolites that are undetectable by conventional neuroimaging thereby holding potential to identify mTBI patients that could benefit from specific neuropsychiatric and cognitive rehabilitation [19]. Brain energy metabolism is altered after TBI due to posttraumatic ischemia with mitochondrial dysfunction and loss

of neuronal integrity with increased cell membrane turnover. In vivo MRS is an MRI technique that can detect nuclei with spins such as ^1H , an abundant by-product of cellular respiration and brain tissue metabolites. As a noninvasive and safe technique, MRS is available on clinical MR scanners (1.5 and 3.0 T) without ionizing radiation [20]. This method holds the potential to identify compromised brain metabolism, but evidence after mTBI is scarce [21]. Key metabolites such as *N*-acetyl-aspartate (NAA), glutamate (Glu), and choline (Cho) have the potential to capture the dynamic and fuller picture of the secondary changes occurring in the injured brain, while creatine (Cr) is widely recommended as a reference marker (Table 1). However, its current application in TBI patients is purely analytical [22].

As it stands, there is currently no clinically available objective biological tool for mTBI classification beyond the GCS – albeit a major goal of the large TRACK-TBI and CENTER-TBI trials. Thus, early detection of compromised brain metabolism as marker for cognitive dysfunction or even the risk for posttraumatic dementia would be highly appreciated by clinicians to provide patients and their families with more reliable information, meanwhile creating a standardized protocol for TBI diagnosis [4, 23, 24]. With these conclusions in mind, we performed a systematic review and meta-analysis to elucidate if MRS might be suitable to identify changes in brain metabolism in adult mTBI patients having a negative CCT and/or MRI brain scan during the acute or subacute postinjury phase in comparison to their age- and sex-matched healthy controls (HC).

Materials and Methods

This systematic review and meta-analysis were performed according to the PRISMA guidelines for systematic reviews and meta-analysis [25, 26]. Eligibility was assessed according to the PICO (patient, intervention, control, outcome) process. We included original data reporting on adult mTBI patients who underwent MRS with an MRI field strength of 1.5 or 3 T within 90 days after injury (acute and subacute phase) and compared those to age- and sex-matched HC. Outcome measures were MRS metabolites measured in different regions of interests (ROIs). mTBI was defined according to the ACRM as an external head and brain impact with a transient or permanent focal neurological deficit and/or any alteration in quantitative or qualitative consciousness that does not exceed (i) a GCS of 13–15 after the first 30 min, (ii) a loss of consciousness of 30 min, and (iii) posttraumatic amnesia <24 h [5, 6].

Inclusion and Exclusion Criteria

We included original, English, and peer-reviewed research articles reporting on controlled studies using MRS in adult patients

Table 1. Magnetic resonance spectroscopy (MRS) metabolite characteristics and changes during the acute/chronic stages after TBI [20]

Metabolite	Spectrum peak, ppm	Normal conc. range, mmol/L	Synthesis/precursor molecules	Role in the healthy brain	Acute changes following TBI	Chronic changes following TBI
N-acetyl-aspartate (NAA)	2.02	7.5–17	Aspartate + acetyl-CoA with L-aspartate N-acetyltransferase enzyme or N-acetylaspartylglutamate split by N-acetylated- α -linked-aminodipetidase enzyme	Myelin lipid synthesis, mitochondrial function, neuronal viability, osmoregulation	Rapid decrease 48 h after TBI; disruptions to neuronal NAA occur due to mitochondrial dysfunction; increased hydrolysis of NAA to provide acetate for myelin repair	NAA recovery in less severe injuries within days after TBI; slow or no recovery in severely injured brain concomitant with hypoxia or hypoperfusion and consecutive irreversible physical and metabolic damage
Glutamate (Glu)	2.3	6.0–12.5	Released from vesicles and converted to Gln and cycled back to neurons in the Glu-Gln cycle; often measured through MRS as the combination of both molecules expressed as the “Glx” signal	Main excitatory neurotransmitter in the central nervous system	Elevated Glx signal early after TBI in occipital gray and parietal white matter	Acute increase in Glu levels correlated with poorer long-term outcome 6–12 months after TBI
Choline (Cho)	3.24	0.5–2.5	Free choline + phosphocholine + glycerophosphocholine	Marker for cell membrane turnover or cell destruction in aggressive brain tumors and neurodegenerative diseases	Increased levels due to cellular damage and membrane loss	Cho levels remain elevated up to 3 months after moderate/severe TBI. Increased Cho levels during the acute phase correlated with poorer long-term outcome at 6–12 months
Creatine (Cr)	3.02	n/a ^a	Phosphorylated from phosphocreatine (PCr)	Marker for brain energy metabolism, ATP synthesis, and regeneration	Consensus that it remains at stable levels following TBI, and thus used as a reference in metabolite ratios	n/a ^b

ATP, adenosine triphosphate; conc., concentration; Gln, glutamine; Glx, glutamate + glutamine; ppm, parts per million; TBI, traumatic brain injury.
^a No consensus on the accepted value.
^b Remains stable over time and is currently used as reference marker.

(≥ 18 years) having experienced a single mTBI (ACMR criteria) during the acute or subacute phase after the brain impact. Data were included from patients with no clinical need for a brain scan or with a negative CCT or MRI. At least 1 of the following metabolites or metabolite ratios had to be measured: NAA, Cho, Glu, Cr, glutamine (Gln), myoinositol (mIns), NAA/Cr, Cho/Cr, Glx (Glu/Gln)/Cr, and mIns/Cr. Exclusion criteria were any brain pathology in a CCT and/or MRI scan, MRS performed beyond 90 days after TBI, children, patients with repetitive concussions with a previous TBI or any premorbidly diagnosed neurobehavioral (e.g., depression or bipolar disorder) or neurological comorbidity (e.g., seizures and brain tumors), non-peer-reviewed articles, conference abstracts, case reports, book chapters, non-controlled studies, interventional studies, animal studies, and non-English language articles.

Search and Data Selection

A comprehensive literature search was conducted from the first edition of electronic databases until January 31, 2020, specifically through PubMed (1901 to present), ScienceDirect (2000 to present), Cochrane Library (1991 to present), and Scopus (1980 to present; Fig. 1). Relevant reviews and their supplementary material were included in full-text screening to check analyzed studies for eligibility as well as their reference lists to ensure that the most possible studies were identified. Search terms are available in the online supplementary Table S1 (for all online suppl. material, see www.karger.com/doi/10.1159/000508098). After removing duplicates, all articles were screened independently in a blinded standardized manner by 2 reviewers (A.E. and M.H.-S.). Disagreements on inclusion between reviewers were resolved for final decision through a detailed discussion and an additional reviewer (K.R.).

Risk of Bias Assessment

A risk of bias assessment was performed using a modified version of the ROBINS-I tool [27]. Evaluation of confounding, selection, classification of intervention(s), missing data, measurement

of outcome(s), and selection of the reported result biases were assessed with either low, high, or unclear risk judgments, and the GRADE assessment was used to evaluate the level of evidence across included studies [28].

Statistical Analysis

Demographic data (sex and age) and basic characteristics (MRS time point after TBI, metabolites, ROIs, and MRI field strengths) are presented, and data are given as means \pm SD and/or ranges. For the meta-analysis, metabolites were analyzed if applied in at least 2 out of the 4 included studies. Statistical analysis and graphs were performed using Review Manager Software 5.3 (Copenhagen, Denmark) and R (R Core Team, Vienna, Austria), respectively [29]. Effect size (ES) and 95% confidence intervals (CIs) of each defined MRS metabolite (Table 1), namely NAA, Glu, Cho, and Cr, were calculated using a random-effects model according to DerSimonian and Laird [30].

Results

The database search returned 5,395 articles and further 22 articles were identified from relevant reviews or reference lists (Fig. 1). After removing duplicates, 5,276 articles were screened by title and abstract. Thereafter, 94 full-text articles were assessed for eligibility. Two articles were eligible for the systematic review, below indicated as study 1 by Gasparovic et al. [24] and as study 2 by Sivák et al. [31], and 4 articles were eligible for the meta-analysis, namely study 1 [24], study 2 [31], study 3 by Veeramuthu et al. [32], and study 4 by Cohen et al. [33]. Studies 3 and 4 were not eligible for the systematic review [32, 33] due to the lack of subgroup analysis of CCT-negative patients.

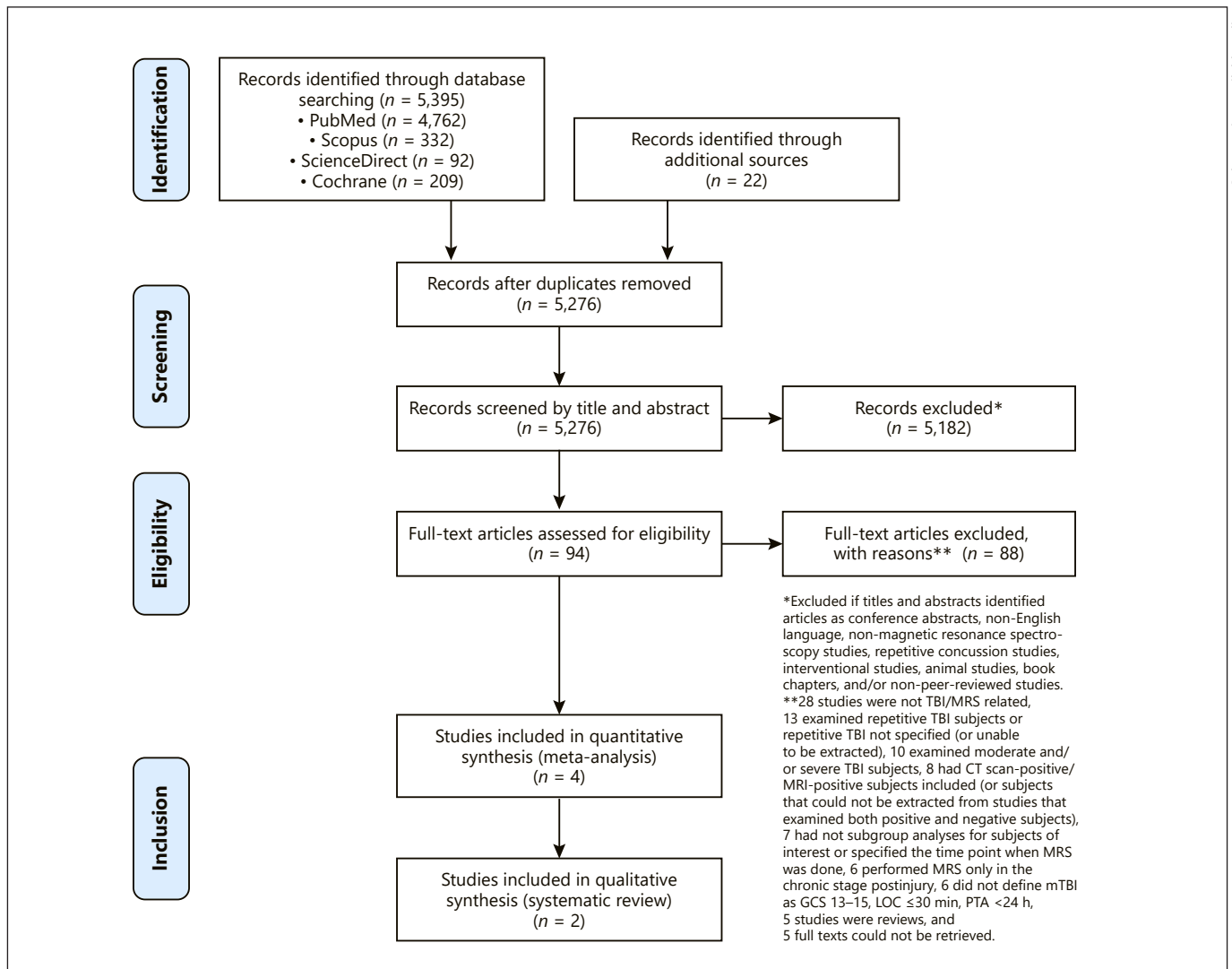


Fig. 1. PRISMA flowchart depicting included full-text articles. Two and 4 out of 94 full-text articles were included in the systematic review and meta-analysis, respectively.

Systematic Review

The 2 included studies analyzed a total of 31 mTBI patients (23 males, 8 females) and 31 sex- and aged-matched HC (24 males, 7 females) with a mean age of 31 and 30 years, respectively (Table 2). The mean time between TBI and MRS was 6.4 days (range 2.7–19 days). Metabolites quantified in both studies were NAA or NAA + NAA glutamate, NAA/Cr, Cho, and Cr. Authors analyzed the following ROIs: study 1 investigated (i) the white matter (WM) between anterior cingulum, medial frontal gyrus, and superior longitudinal fasciculus, (ii) gray matter (GM) in the interhemispheric fissure, and (iii) the splenium, while study 2 analyzed (iv) the right frontal lobe, (v) the left frontal lobe, and (vi) the pons (Table 2).

Gasparovic et al. [24] (study 1) found a nonsignificant Cho change in different ROIs. In detail, Cho was slightly increased compared to HC in the interhemispheric fissure (GM) and in the splenium, whereas it was decreased in the WM, that is, 1 cm above the lateral ventricles. Cr was significantly increased in the WM and splenium, and nonsignificantly decreased in the GM. Glx was significantly decreased in the GM, and nonsignificantly increased in the WM. No significant effect was seen for NAA in the above-mentioned ROIs and time points, namely 3–19 days after mTBI.

Sivák et al. [31] (study 2) observed a significantly lower NAA concentration in the right and left frontal lobes. Lower NAA/Cr and Cho/Cr ratios were found in the right

Table 2. Demographics and patient characteristics of publications included in the systematic review

Sample size	Sex, mTBI/HC		Age (mTBI/HC), years		Time after-TBI		ROI	Metabolites	Reason for ROIs	MRI field strength	Objective	Significant results
	mTBI	HC	male	female	mean	range						
Gasparovic et al. [24], 2009	10	9	4/4	6/5	29.0±9.7 27.6±9.1	21–49 21–49	days	3–19	10.7	days	GM and WM: WM between anterior cingulum, medial frontal gyrus and superior longitudinal fasciculus and GM of interhemispheric fissure Single ¹ H-MRS: splenium	Using ¹ H-MRS and ¹ H-MRSI to compare brain metabolites within 3 weeks after mTBI to levels of HC healthy controls Cr in WM and splenium higher and Glx in GM lower after mTBI compared to healthy controls
Sivák et al. [31], 2014	21	22	19/20	2/2	33.2±12.4 31.5±11.7	20–58 20–55	2 days	32.9– 64.9 h	2 days	1.5 T	Determine the association of clinical or neuropsychological performance with ¹ H-MRS metabolite changes during the acute phase of mTBI	NAA in left frontal lobe and NAA/Cr in right frontal lobe lower after mTBI versus HC
Total	31	31	23/24	8/7	31.1 29.6	20–58 20–55	2.7– 19 days	6.4 days	n/a	n/a	n/a	n/a

mTBI, mild traumatic brain injury; HC, healthy controls; ROI, region of interest; ¹H-MRS, proton magnetic resonance spectroscopy (MRS); ¹H-MRSI, single voxel ¹H-MRS and proton MRS imaging; NAA, N-acetyl-aspartate, NAAAG, NAA glutamate; Cho, choline; Cr, creatine; Glu, glutamate; Gln, glutamine; GPC, glycerophosphocholine; Ins, inositol; MM09, macromolecules at signals around 0.9 ppm; MM09+Lip09, MM09 and lipids signals at around 0.9 ppm; MM20, macromolecules at signals around 2.0 ppm; MM20+Lip20, MM20 and lipid signals at around 2.0 ppm; PCr, phosphoCho; PCr, phosphoCr; GM, gray matter; WM, white matter; dIPFA, dorsolateral prefrontal area; ARAS, ascending reticular activating system; n/a, not available/not applicable.

^a No raw data available.

Table 3. Demographics and patient characteristics of the publications included in the meta-analysis

Sample size	Sex (mTBI/HC)		Age (mTBI/HC), years		Time after TBI		Metabolites	ROI	Reason for ROIs	MRI field strength	Objective	Data used for meta-analysis	
	mTBI	HC	male	female	mean ± SD	range							
Gasparovic et al. [24], 2009	10	9	4/4	6/5	29.0±9.7 27.6±9.1	21–49 21–49	days	3–19	10.7	days	GM and WM: reasons for ROIs not given Splenium: to observe metabolic changes in a region susceptible to axonal injury after TBI	Using ¹ H-MRS and ¹ H-MRSI to compare brain metabolites within 3 weeks after mTBI to levels of HC	WM: NAA + NAAAG, Cho, Cr, Glu
Sivák et al. [31], 2014	21	22	19/20	2/2	33.2±12.4 31.5±11.7	20–58 20–55	2 days	32.9– 64.9 h	NAA, NAA/Cr, NAA/Cho, Cho, Cho/Cr, Cr	Single ¹ H-MRS: right and left frontal lobe (dIPFA), pons	dIPFA and ARAS are typically affected after TBI and their involvement correlates with posttraumatic cognitive dysfunction in terms of executive functions, processing speed, working memory, and attention deficits or loss of consciousness, respectively	Determine the association of clinical or neuropsychological performance with ¹ H-MRS metabolite changes during the acute phase of mTBI	Left frontal lobe: NAA, Cho, Cr
Veeramuthu et al. [32], 2018	12	13	10/8	2/5	28.3±7.3 27.2±4.9	n/a	n/a	10 h (SD: 4.3)	NAA, NAA+NAAAG, Glu, GPC, Ins, GPC+PCCh, Cr+PCr, Glu+Gln, MM09, MM20, MM09+Lip09, MM20+Lip20	Single ¹ H-MRS of WM in mTBI patients: frontal, parietal, occipital lobe; controls: frontal, and parietal lobe	n/a	Investigate the early neurometabolic changes after mTBI versus HC	All regions: NAA, Glu
Cohen et al. [33], 2007	7	7	3/3	4/4	33.3±11.3 34.6±6.2	25–41 27–41	1 day– 1.2 months	8.9 days	NAA	Whole brain	n/a	Quantify the global decline in the neuronal marker NAA after mTBI	Whole brain: NAA
Total	50	51	36/35	14/16	31 30.2	n/a	n/a	5.5 days	Overlap: NAA (+NAAAG), Glu, Cho, Cr	Overlap: frontal lobe	n/a	n/a	n/a

For further information see legend to Table 2.

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Fig. 2. *N*-acetyl-aspartate (NAA) is a marker for neuronal density and integrity. NAA moderately decreases after mTBI in comparison to HC with an overall ES of -0.49 (95% CI -1.08 to 0.09), thus representing a moderate effect. NAA was analyzed in the WM 1 cm above the lateral ventricles (study 1), in the left frontal lobe (study 2), or in all brain regions (studies 3 and 4). Sivák et al. [31] (study 2) compared 21 mTBI patients to 22 HC and elucidated a significant decrease in NAA ($p = 0.002$) in the left frontal lobe when measured 2 days (mean) after mTBI.

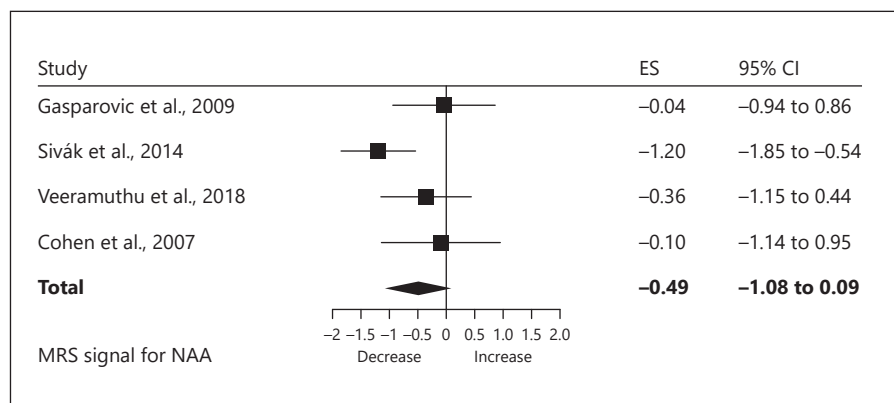
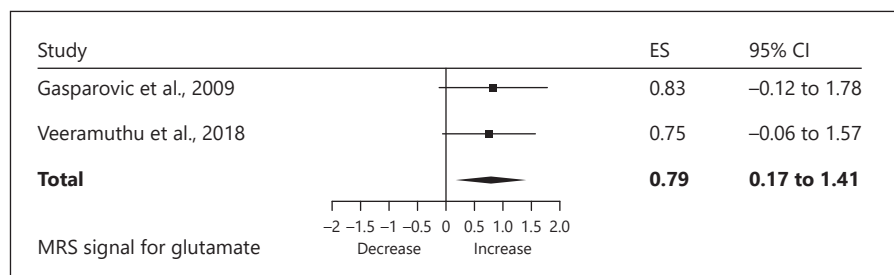


Fig. 3. Glutamate (Glu) is a marker for disturbed brain metabolism. Two out of the 4 included studies (studies 1 and 3) measured increased Glu in the WM 1 cm above the lateral ventricles at 10 h (mean) and in the whole brain 10.7 days (mean) after mTBI, respectively. The overall effect size was strong with 0.79 (95% CI 0.17 – 1.41).



frontal lobe. After Bonferroni's adjustment to correct for multiple comparisons, NAA in the left frontal lobe and NAA/Cr in the right frontal lobe remained significantly lower in patients after mTBI compared to HC. Absolute Cho and Cr concentrations in the left frontal lobe tended to be lower in mTBI patients.

Meta-Analysis

Four studies were included in the meta-analysis analyzing 50 mTBI patients (36 males, 14 females) and 51 HC (35 males, 16 females) with a mean age of 31 and 30 years, respectively. Patients received MRS 5.5 days (mean) after the brain impact (Table 3). Of study 3 [32] a subgroup of CCT-negative mTBI patients ($n = 12$) and of study 4 [33] a subgroup of 7 mTBI and HC were eligible for inclusion. ROIs and their rationales as well as metabolites are detailed in Table 3. Study 2 analyzed the left and right frontal lobes separately, of which we used the data from the left frontal lobe and thereby avoided an overrepresented analysis [31]. Regarding measured metabolites, NAA was analyzed across all 4 studies, while Glu, Cho, and Cr overlapped in 2 out of the 4 studies. MRS sequences, the localization of voxel placement, and voxel sizes differed across all 4 studies or were not given. Studies 1–3 used the PRESS sequence. Study 4 used nonlocalizing proton MRS. Voxel sizes or vol-

umes were $1 \times 1 \times 1$ cm (study 1), 12 mL (cortex), and 9 mL (upper brainstem) (study 2), $19 \times 19 \times 19$ mm (study 3), or were not specified (study 4). Within and across studies, risk of bias was low (online suppl. Table S2).

N-Acetyl-Aspartate/*N*-Acetyl-Aspartyl-Glutamate

Across all 4 studies, NAA showed a moderate decline with a moderate overall ES of -0.49 (95% CI -1.08 to 0.09) in 50 mTBI patients compared to 51 HC (Fig. 2). This result was mainly based on the findings of study 2, in which NAA was analyzed in the left frontal lobe in 21 mTBI patients (ES -1.2 ; 95% CI -1.85 to -0.54). In studies 1, 3, and 4, there were no significant differences in NAA concentrations between mTBI patients and HC.

Glutamate

Studies 1 and 3 found increased Glu, which was primarily measured in the WM of the parietal lobe in mTBI patients compared to HC with an overall strong ES of 0.79 (95% CI 0.17 – 1.41 ; Fig. 3).

Choline

Studies 1 and 2 reported on decreased Cho in mTBI patients with a weak overall ES of -0.31 (95% CI -0.81 to 0.19 ; Fig. 4).

Fig. 4. Choline (Cho) is a marker for increased cell membrane turnover, and increased levels in MRS detect cellular damage after mTBI. Two out of the 4 included studies (studies 1 and 2) measured increased Cho in the WM 1 cm above the lateral ventricles at 10 h (mean) and in the left frontal lobe 2 days (mean) after mTBI, respectively. The overall effect size was moderate with -0.31 (95% CI -0.81 to 0.19).

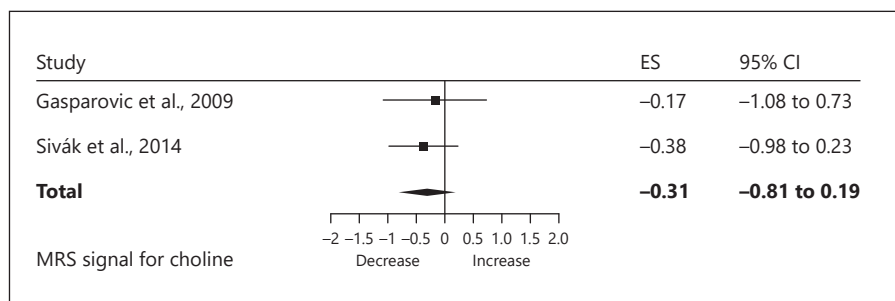
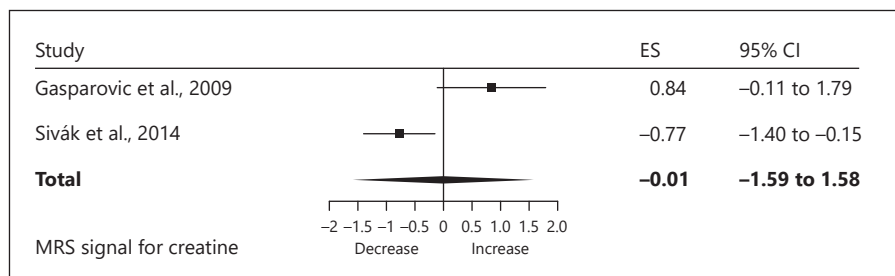


Fig. 5. Creatine (Cr) is currently suggested as a reference marker for MRS. Two out of the 4 included studies (studies 1 and 2) measured contradictory Cr results in the WM 1 cm above the lateral ventricles 10 h (mean) and in the left frontal lobe 10.7 days (mean) after mTBI, respectively. The overall effect size was very low -0.01 (95% CI -1.59 to 1.58), which suggests that Cr is not a suitable reference marker after mTBI.



Creatine

Study 1 showed a significant increase in Cr, which was measured in the WM, that is, 1 cm above the lateral ventricles with an ES of 0.84 (95% CI -0.11 to 1.79 ; Fig. 5). Study 2 reported a nonsignificant Cr decrease in the left frontal lobe with an ES of -0.77 (95% CI -1.4 to -0.15). These contradictory results led to an overall ES for Cr of -0.01 (95% CI -1.59 to 1.58).

Discussion

In this systematic review and meta-analysis, 2 and 4 out of 5,417 identified records were included, respectively. The most crucial findings were alterations in NAA and Glu concentrations using MRS at 3 or 1.5 T within 90 days after injury, i.e., during the acute and subacute phase after a single mTBI in adults having a CCT/MRI-negative brain scan. Precisely, lowered NAA and increased Glu concentrations were found in mTBI patients compared to HC, indicative for loss of neuronal integrity and disturbed brain metabolism [20, 24, 34]. Cho slightly decreased in mTBI patients, which differs to the amplified Cho concentrations in patients with neurodegenerative diseases and brain tumors as a hallmark of cell membrane turnover [20]. In contrast, there is evidence that Cho increases over time when measured in the WM but not in the GM up to 55 days after mTBI [35], which indicates that the axonal

pathology and precise definitions of where and when to measure metabolites are needed. However, the relevance of the overall decreased Cho measured in the GM and WM within study 1 and 2 during the acute and subacute phase after mTBI remains unclear and might be associated to the function of Cho as a precursor molecule for myelin. Thus, we suggest to repetitively measure Cho to elucidate its function over time after mTBI. Results on Cr concentrations in mTBI patients were contradictory and potentially attributed to different field strengths and ROIs. Moreover, a recent longitudinal study demonstrated that higher Cr levels in the subacute stage were positively correlated to cognitive impairment during the chronic stage after mTBI when measured in the centrum semiovale, thus in the WM [36]. Kirov et al. [35] found increasing Cr levels over time when measured in the WM but not in the GM up to 55 days after mTBI, which again emphasizes the axonal pathology after mTBI. In summary, it remains doubtful that Cr is a suitable reference marker following mTBI as previously suggested [20].

When interpreting the current results, different time points of MRS after mTBI must be considered especially as the results of NAA and Cho are mainly driven by study 2 (Sivák et al. [31]), which measured brain metabolites within 33–64 h after mTBI. Thus, early MRS after mTBI could be favorable in detecting relevant metabolic changes, and we suggest bearing this in mind for future investigations.

We chose strict inclusion criteria investigating mTBI patients with negative routine brain scans that are often underdiagnosed yet still exhibit posttraumatic symptoms [37]. Early diagnosis of those presumably mildly brain-injured patients at risk for long-term consequences is critically relevant as longitudinal follow-up studies underline continued symptoms, including chronic fatigue, psychiatric disorders, and unsatisfied quality of life in one third of patients [38–41]. A recent meta-analysis revealed that MRS might have limitations for use in mTBI patients [21], which is contradictory to our findings possibly due to a greater heterogeneity of their study population. In detail, this meta-analysis defined broader inclusion criteria analyzing mTBI not strictly using the ACMR guidelines and additionally included postconcussion patients during the acute, subacute, or chronic phase with or without positive brain scans. This meta-analysis found no brain metabolite changes in these mixed mTBI and postconcussion patients [21], most probably accentuating the different pathophysiology after concussion and single mTBI.

Integrating the current evidence, there is a need for standardized temporal and spatial MRS protocols for future research of mTBI patients.

MRS and the Link to Neurodegeneration

MRS exploits metabolic alterations in neurodegenerative diseases and brain tumors [42–45], but evidence following mTBI is sparse. Animal studies using MRS after experimental TBI suggest Gln, pyruvate, glycerol, and phosphocholine as suitable metabolites to detect diffuse axonal injury and posttraumatic neurodegeneration [46–50]. However, patients with early signs of dementia have exhibited reductions in Glu and NAA [51], suggesting that these metabolic alterations are not specific for TBI, but might point to the posttraumatic pathway and link to neurodegeneration. Moreover, NAA, Glx, and mIns correlated with cognitive outcomes in pediatric TBI patients of all severities [52]. Repetitive sport-related concussions result in chronic neuroinflammation and neurodegeneration, and MRS was previously suggested as a potential biomarker to detect those neurometabolic changes and cognitive impairment [53–57]. Microglial activation and chronic neuroinflammation are discussed as triggers for posttraumatic neurodegeneration [3, 58–60], and mIns, a metabolite involved in osmoregulation and astrocyte activity, was elevated after mTBI and thus might be a promising marker [61]. Moreover, future studies should focus on the evident metabolic changes in mIns, mIns/Cr, and Glx/Cr in the putamen and, thus, in the deep GM not only

involved in motor but also in cognitive and emotional circuits, particularly working memory, cognitive control, category learning, and emotional control, functions often hampered after mTBI [61].

ROIs following TBI

Our systematic review and meta-analysis brought up the heterogeneity of analyzed ROIs following mTBI as the only overlapping ROI was the frontal lobe in 3 out of the 4 studies. In study 2, we included data of the left frontal lobe where NAA was most significantly decreased, and the hampered brain metabolism negatively correlated with neuropsychological performance [31]. Animal studies using MRS following experimental mTBI suggest that the most significant axonal damage is observed in the hippocampus, thalamus, internal capsule, and corpus callosum as early as 2 h after injury [62]. Thus, we suggest these brain regions to be considered in future trials for standardized ROIs after mTBI. To date, it is difficult to draw conclusions from MRS studies because ROIs are not precisely defined and often do not focus on WM well known to be the pathological hallmark of diffuse axonal injury that causes attentional and cognitive deficits after TBI [45].

Limitations

A limited number of articles with small sample sizes were eligible for inclusion. Furthermore, MRI sequences, field strength, voxel placements, and sizes were incongruent between studies, and Cramer Rao Lower bounds cut-offs were only given in study 3. This is necessary as a good signal depends mostly on voxel size, depth, and on the ROI (e.g., signals are less clear at borders with cerebrospinal fluid or air).

Conclusion

The strongest evidence of metabolic changes was found for decreased NAA and elevated Glu concentrations during the acute to subacute phase in adult mTBI patients compared to HC, while Cr might be not suitable as a reference marker in mTBI patients. If these metabolites have the capacity to recognize patients with negative routine brain scans that are at risk for posttraumatic long-term sequelae is still not yet clear. Follow-up studies correlating MRS with cognitive outcome are necessary to elucidate the potential of these metabolites as biomarkers to predict and detect posttraumatic neurodegeneration at an early stage.

Statement of Ethics

This systematic review and meta-analysis were performed according to the PRISMA guidelines for systematic reviews and meta-analysis. Ethical approval for the literature search was not required.

Disclosure Statement

The authors declare no conflicts of interests.

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Author Contributions

A.E. and M.H.-S. contributed equally to data collection, analysis, interpretation, and writing of the manuscript. L.M. provided technical expertise on the MRS topic and helped revise the manuscript. K.R. designed and supervised the entire systematic review and meta-analysis, helped in decision making for study inclusion, and wrote and revised the manuscript. All authors approved the final version of the manuscript.

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